SELDI ANALYSIS

The Need for the Review and Understanding of SELDI/MALDI Data Prior to Analysis (Analyzer Beware)

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SELDI ANALYSIS

WINDOW INTO DISEASES

Plasma Serum Urine

Effusions

Mucous Saliva Fecal Matter

Tissue (PAP; Urinary Sediment)

Bile CSF Sweat

SELDI ANALYSIS

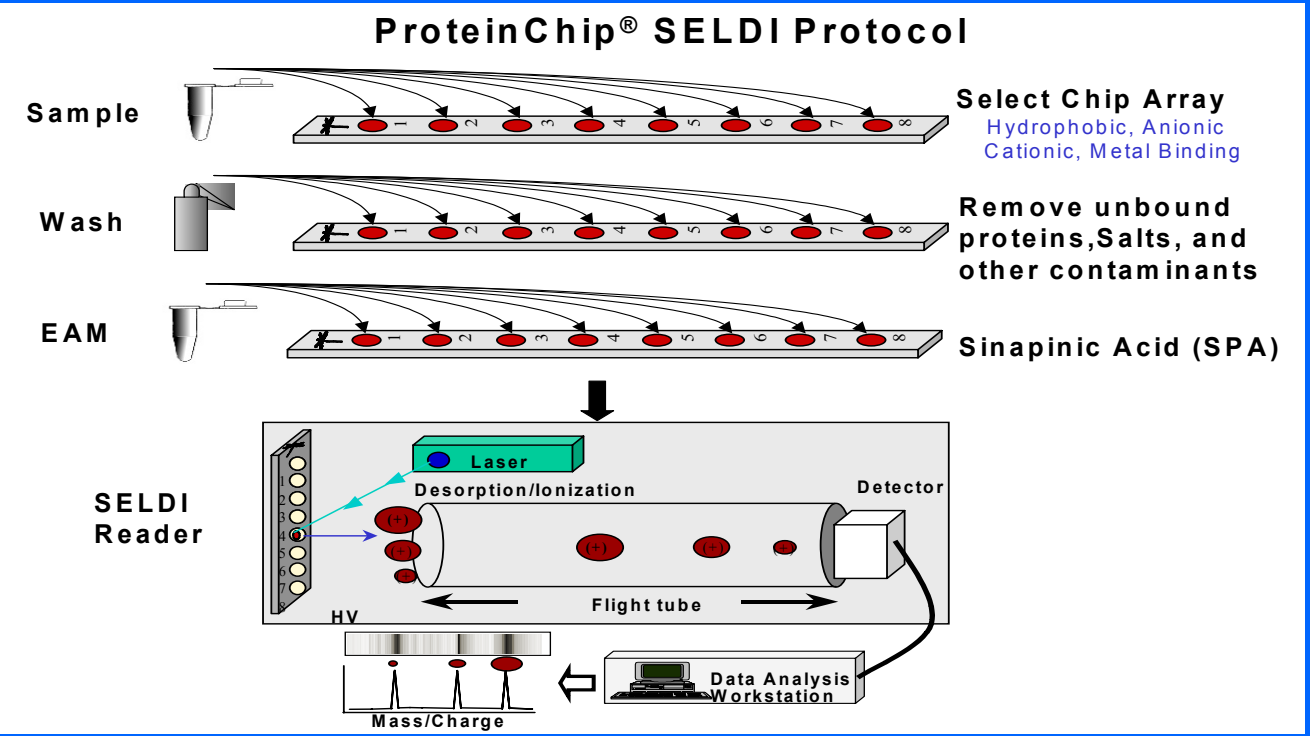
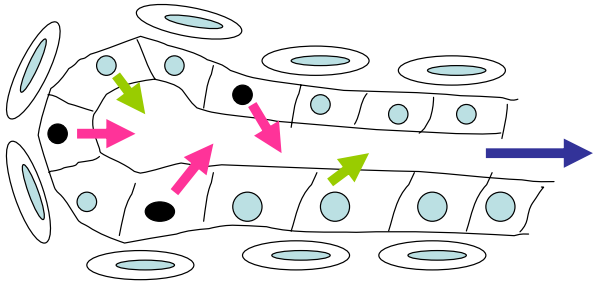
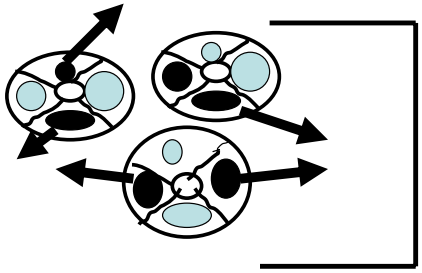


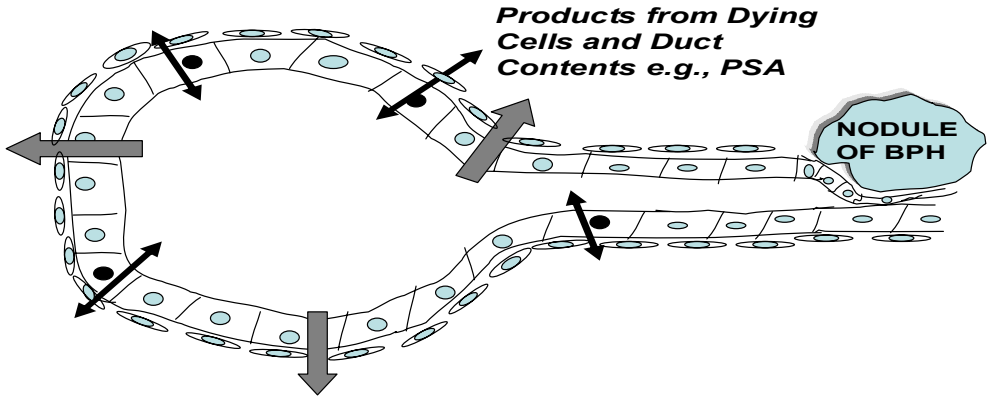
Illustration of SELDI Time-Of-Flight (TOF) Mass Spectrometry. (Modified with permission from Ciphergen Biosystems, Inc.)

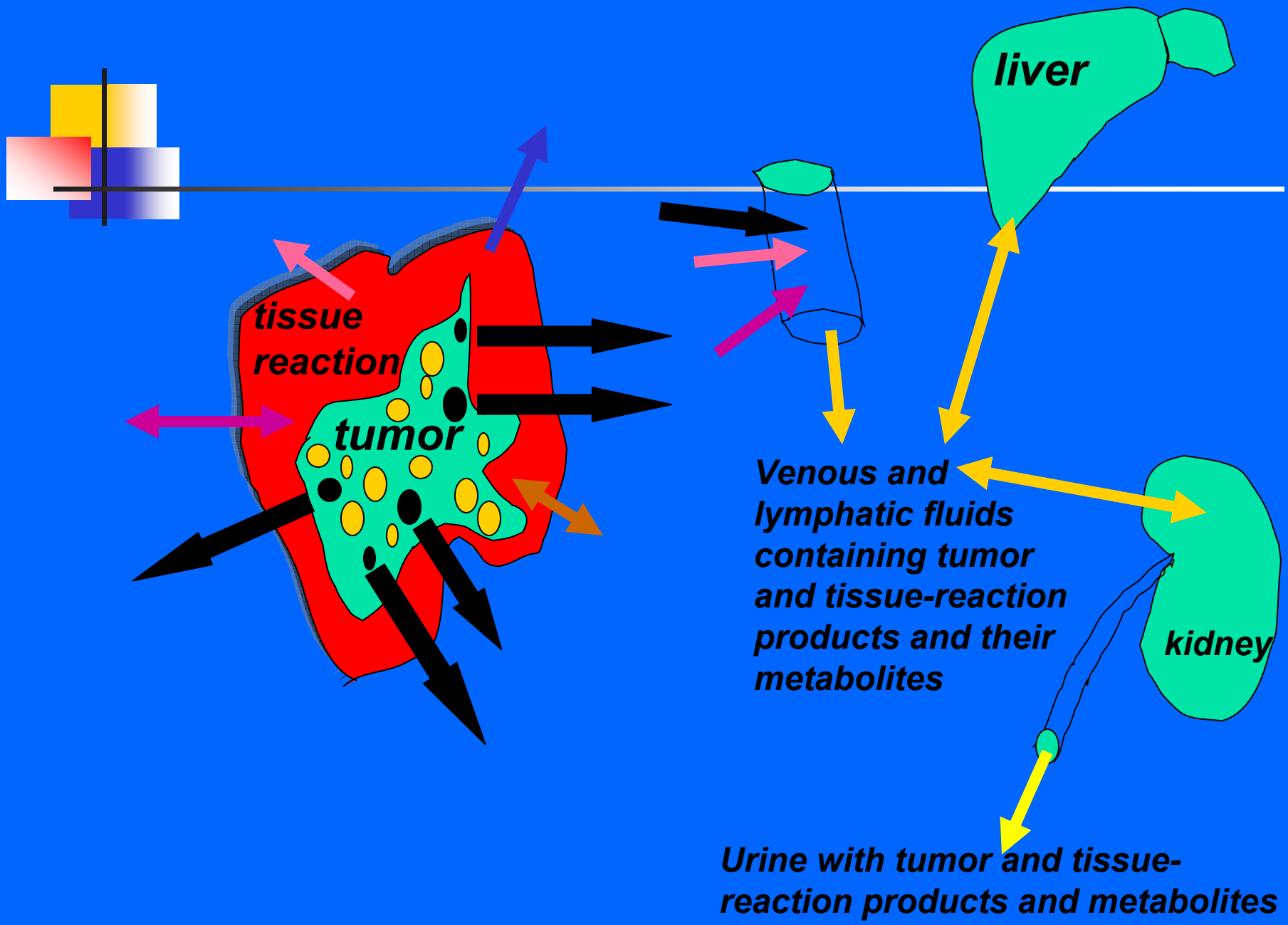


Contents of Duct Including Products of Dying Cells and Living Cells e.g., PSA



Products of Dying Cancer Cells Collect in Interstitial Space and are Absorbed into Vascular and Lymphatic Vessels

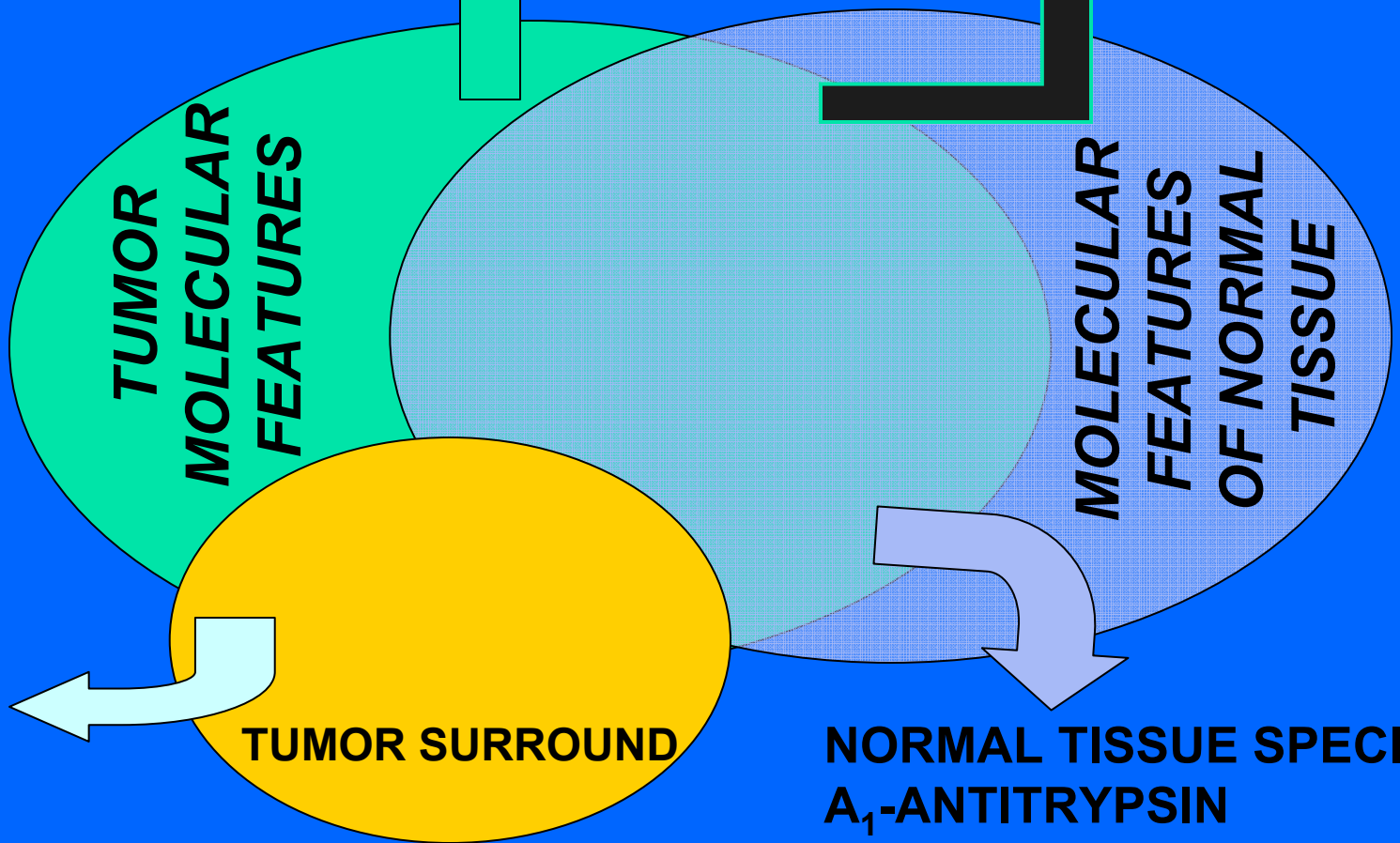



liver
tissue reaction
tumor
Venous and lymphatic fluids containing tumor and tissue-reaction products and their metabolites
kidney
Urine with tumor and tissue-reaction products and metabolites

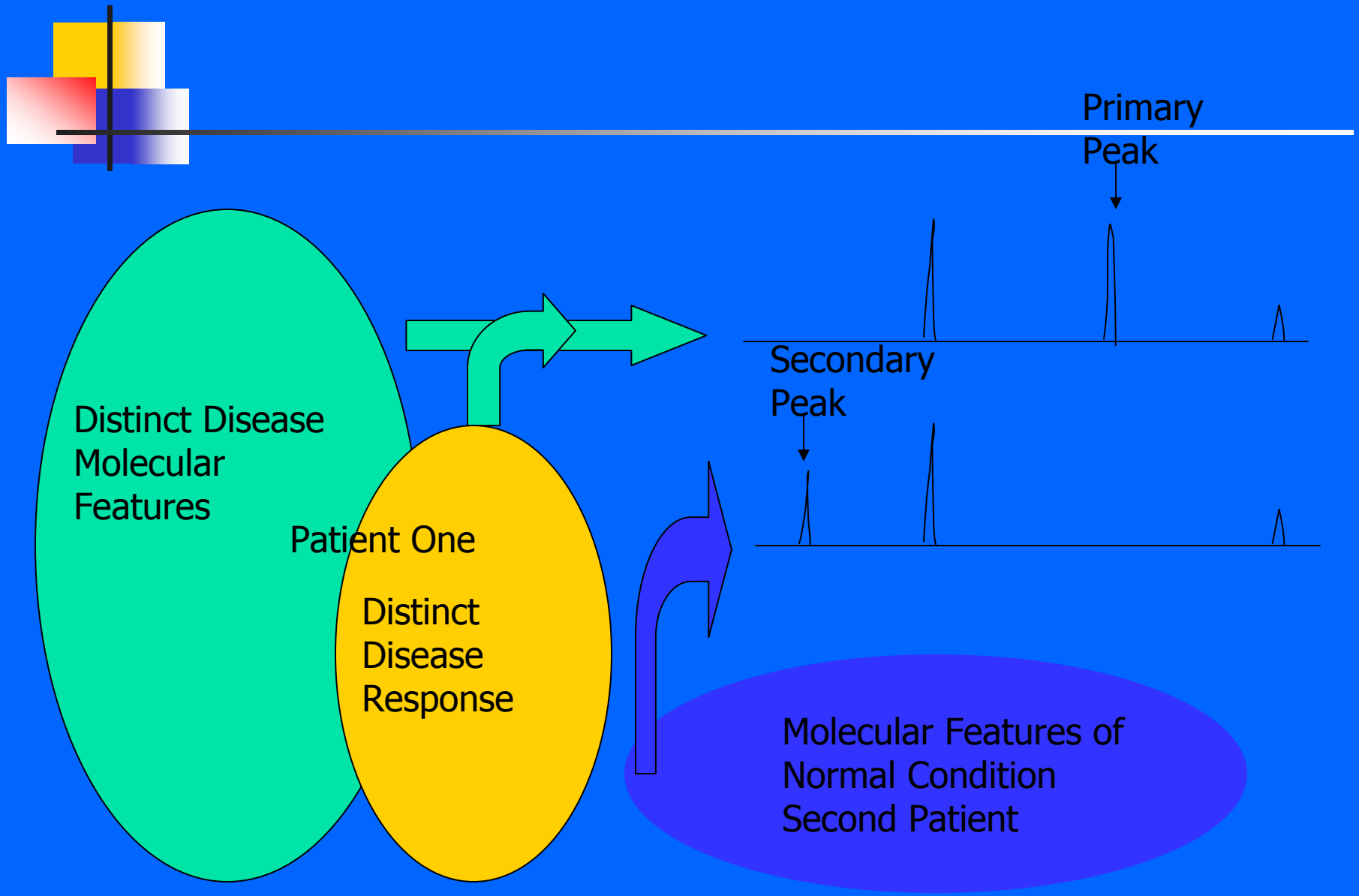
ONCOFETAL TUMOR ANTIGENS AND METABOLITES

CEA
TAG-72
CA125
MUC-2
LEWIS Y

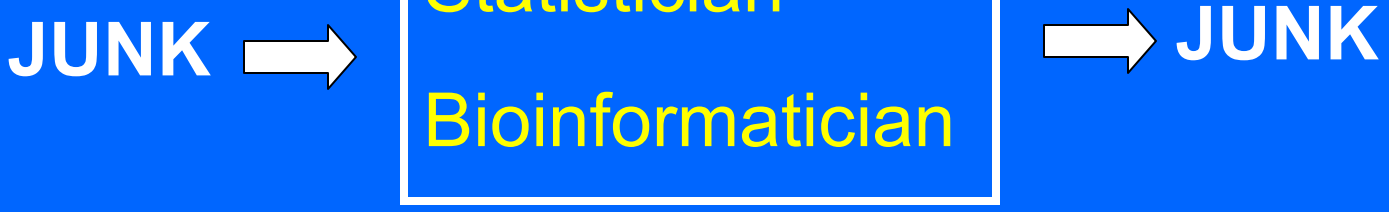
MOLECULES AND METABOLITES OF NORMAL TISSUES - ACTIN

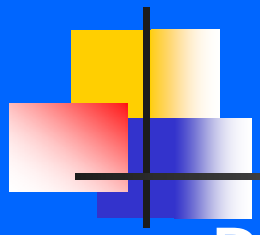


NORMAL TISSUE SPECIFIC - PSA, A_1-ANTITRYPSIN


Primary
Peak
Secondary
Peak
Distinct Disease
Molecular
Features
Patient One
Distinct
Disease
Response
Molecular Features of
Normal Condition
Second Patient

SELDI ANALYSIS

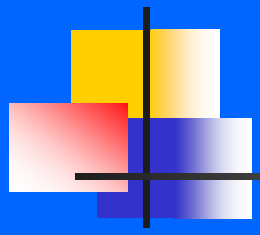




SELDI ANALYSIS

PROBLEMATIC ISSUES IN ANALYSIS

- Experimental Design
- Patient
- Sample
- Protein Chip
- Spectra
- Analytical Approach



SELDI ANALYSIS

- Experimental Design
  - Selecting Cases and Controls
  - Collecting and Processing Samples
  - Performing Assays Without Bias
  - Selecting Optimal Approach to Analysis
  - Avoiding Over-Analysis

SELDI ANALYSIS

PATIENT

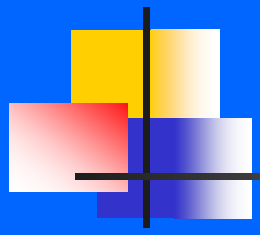
Groups Comparable

Sites

Racial/Ethnic Balance

Homeostatic Balance

No Bias



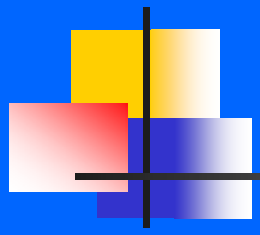
SELDI ANALYSIS

PATIENT

Usually Comparing Disease vs. Control

or

Disease A vs. Condition B vs. Normal



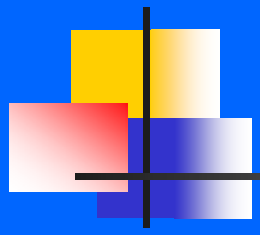
SELDI ANALYSIS

PATIENT

Control Definition

Is Disease Absent?

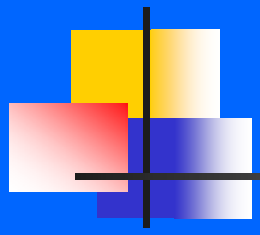
Is There Bias In The Controls?



SELDI ANALYSIS

SAMPLE

- Type
- Collection
- Processing
- Storage
- Transfer



SELDI ANALYSIS

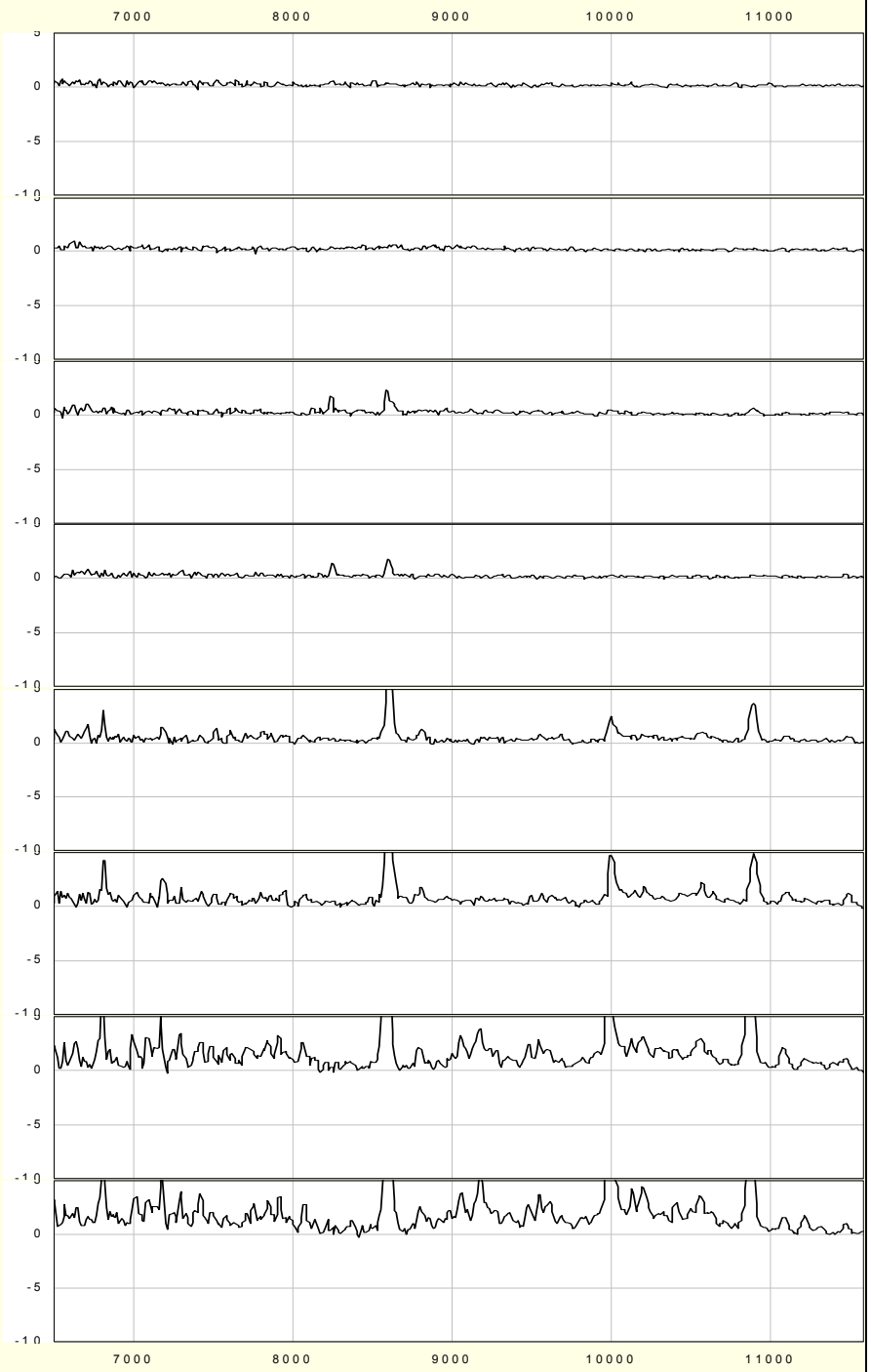
SAMPLE

Type

Serum versus Plasma

Sensitivity- Can Products of Tumors Be Detected

LNCAP CELL LYSATES USING WCX2 ARRAYS



100 Cells per ml

100 Cells per ml

1000 Cells per ml

1000 Cells per ml

10,000 Cells per ml

10,000 Cells per ml

10^5 Cells per ml

10^5 Cells per ml

SELDI ANALYSIS

SAMPLE

Collection

How?

Stress

Container-e.g.., multiple anticoagulants; thus all Plasmas are not the Same

SELDI ANALYSIS

SAMPLE

Processing

Time from Collection to Freezing (Too Restrictive?)

For Consistent Results, Robotic Processing Is Required

SELDI ANALYSIS

SAMPLE

Processing

Removal of Proteins Present in Large Concentrations (e.g., Albumin) May Also Remove Peptides Being Carried by Removed Proteins

SELDI ANALYSIS

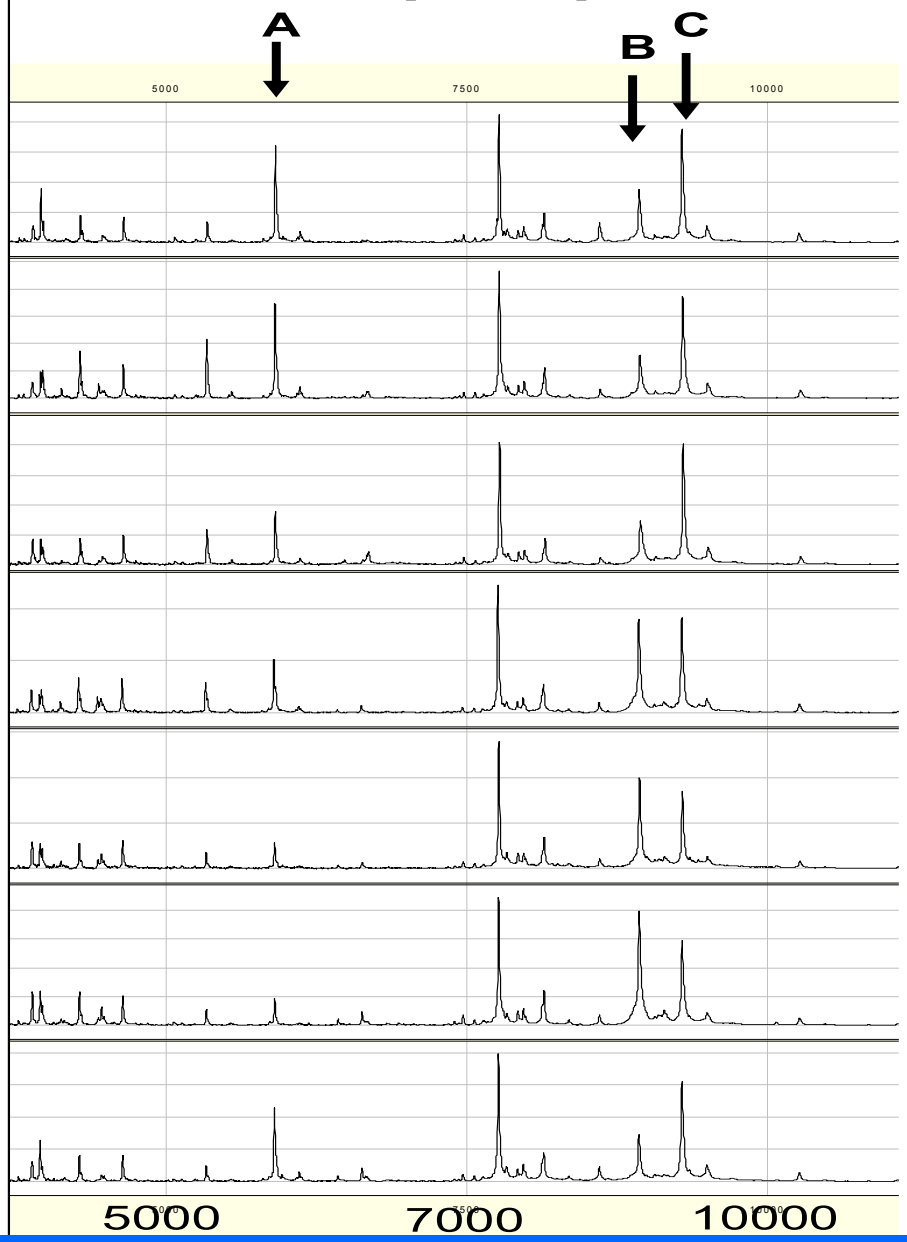
SAMPLE

Storage

Length

Temperature

QC Sample Spectra



Aliquot of Sample (#20) Stored at -80C

Aliquot of Sample (#20) Transferred from -80C and Stored for 3 Months at -20C

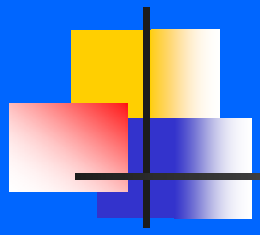
Aliquot of Sample (#20) Stored at -20C for 5 Months

Aliquot of Sample (#20) Stored at -20C for 7 Months

Aliquot of Sample (#20) Stored at -20C for 8 Months

Second Aliquot of Sample (#20) Stored at -20C for 8 Months

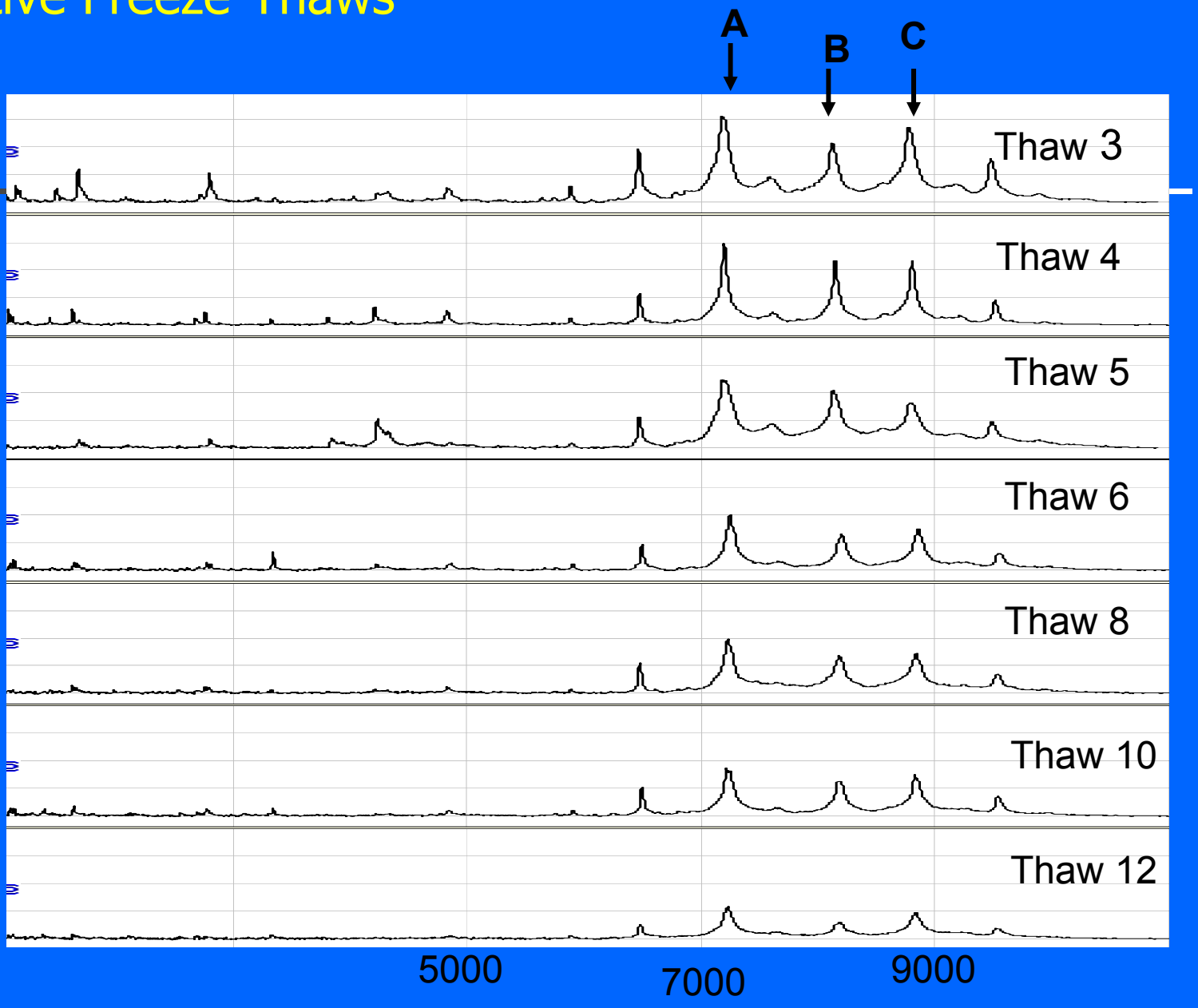
Second Aliquot of Sample (#20) Stored ONLY at -80C for Ten Additional Months



SELDI ANALYSIS

- Transfer
  - Freeze-Thaw Cycles
  - Quantity (Triplicates and Repeat-300 mcl)

Decrease in Protein Intensity in Human Serum due to repetitive Freeze-Thaws



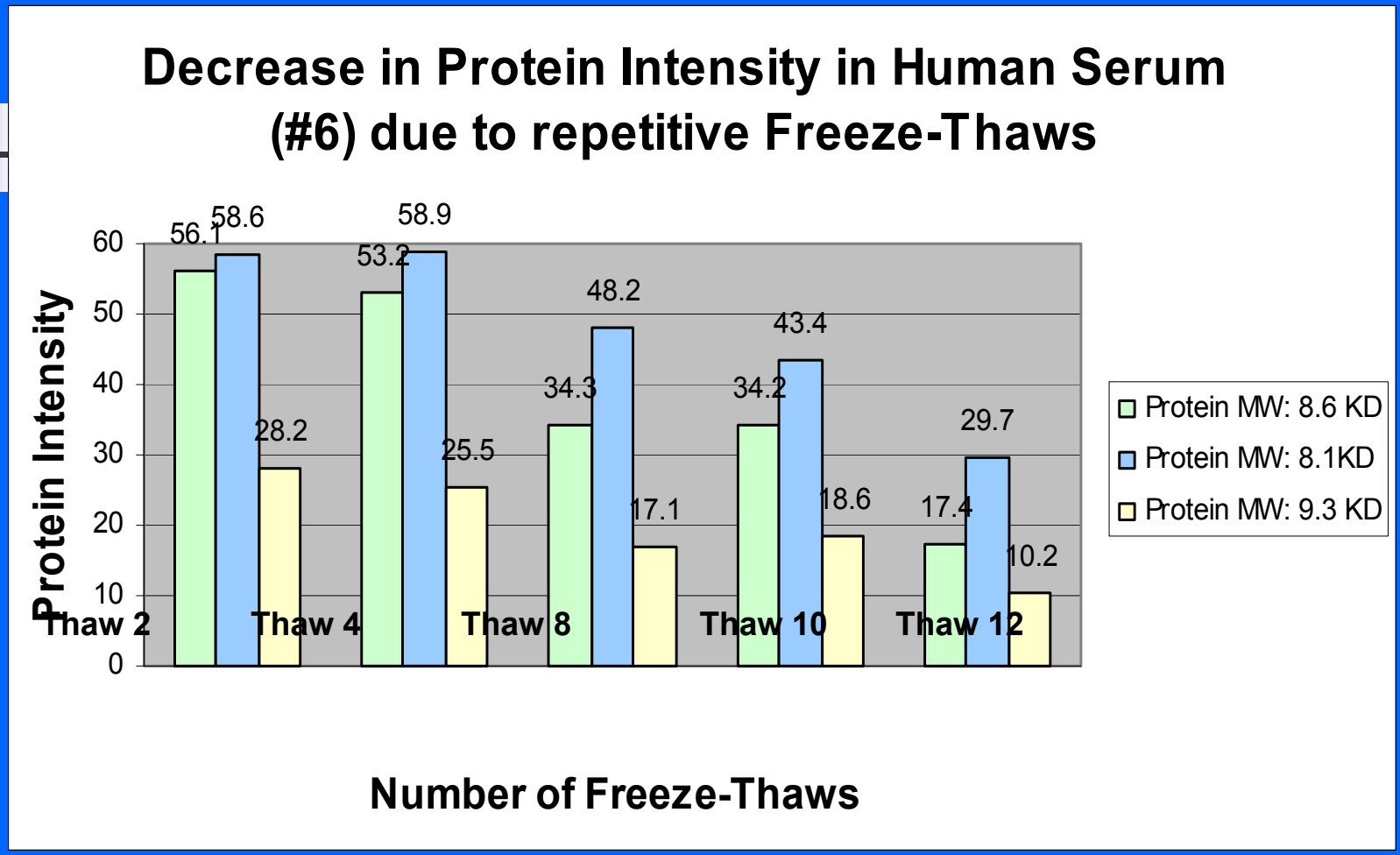
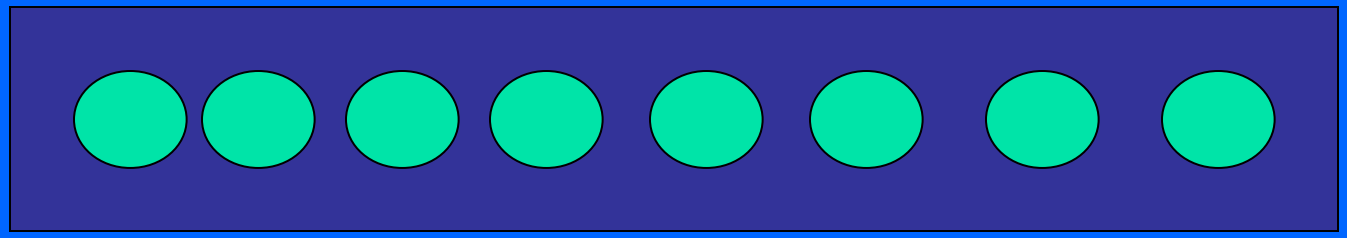


Figure1: *The graph above shows the decrease in protein intensities in three different proteins in one human serum sample. The legend indicates the molecular weight of each protein. The specific intensity values are displayed above each of the bars.*

SELDI ANALYSIS

- Chip Type



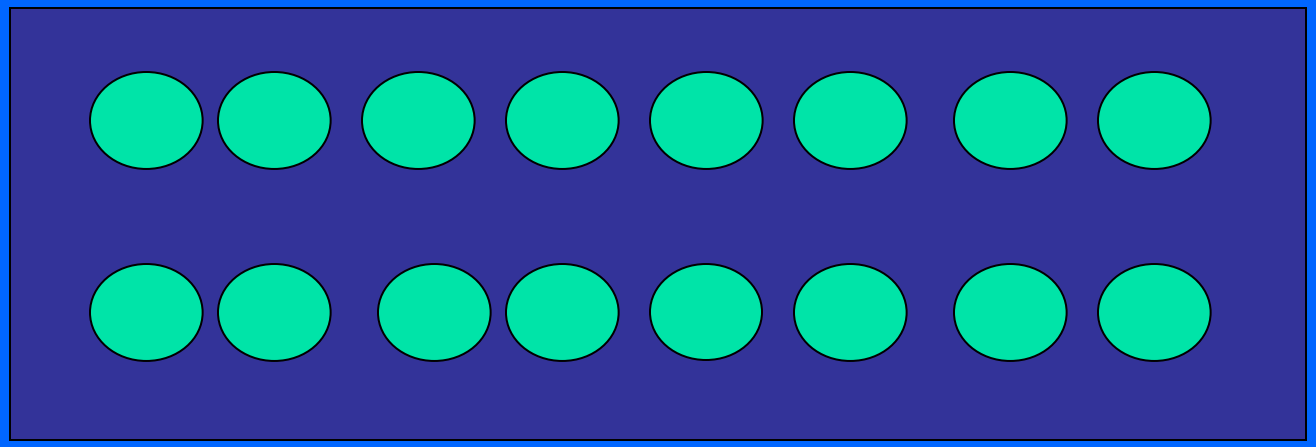
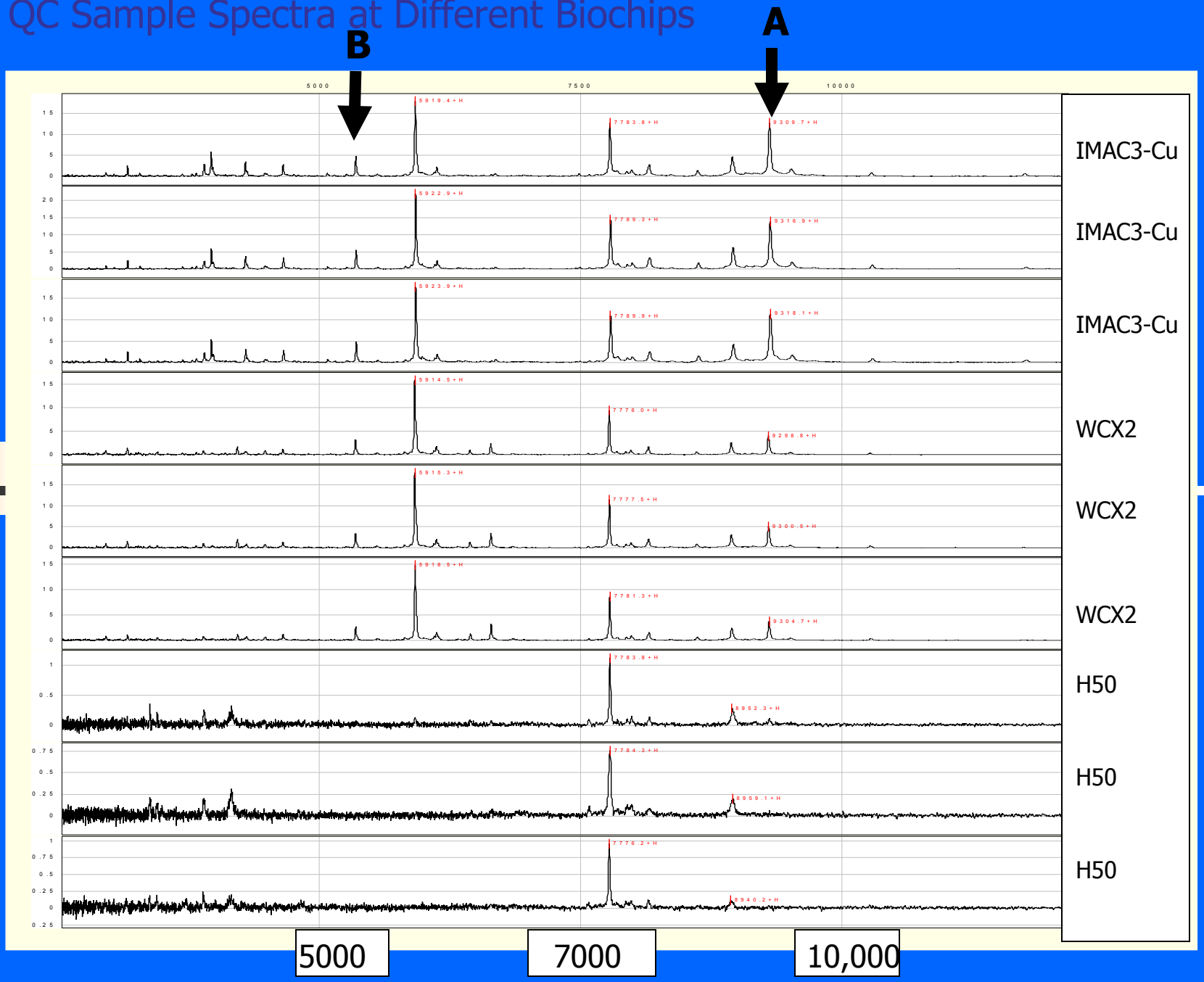


TABLE 1:

Old Designation	Current Chip	Biochemical Action of Surface Chemistry
IMAC3	IMAC30 (with hydrophobic barrier)	Bivalent metals can be attached to the chip. Proteins that bind to these divalent metals (e.g., Cu^{+2}) are bound by the chip.
WCX2	Same (CM10 mimics WCX2 but does not replace it)	This is a weak cation exchange chip. It contains negatively charged (anionic) carboxylate groups that will bind proteins with positively charged areas containing high numbers of lysine, arginine, and/or histidine amino acids.
H4	Same (C16 contains 16 CH_3)	The chip contains multiple chains of 16 methylene groups. This binds molecules that are hydrophobic.
SAX2	Q10 (with hydrophobic barrier)	Strong anion exchanger which is composed of quaternary ammonium groups that are charged positively. This chip will bind proteins/peptides with regions rich in acidic groups, especially regions of peptides high in aspartic and/or glutamic amino acids.
NP1 and NP2	NP20	General protein binding surface with binding of hydrophilic proteins.
PS1 and PS2	PS10 / PS20	Chip designed to bind capture molecules of choices e.g., antibodies, receptors, and nuclei acid binding proteins PS-1 (carbonyl diimidazole groups), PS-2 (epoxy groups). Also the PS-2 has a hydrophobic coating.
SENDID		Incorporates EAM into chip.

QC Sample Spectra at Different Biochips



SELDI ANALYSIS

Protein Chip

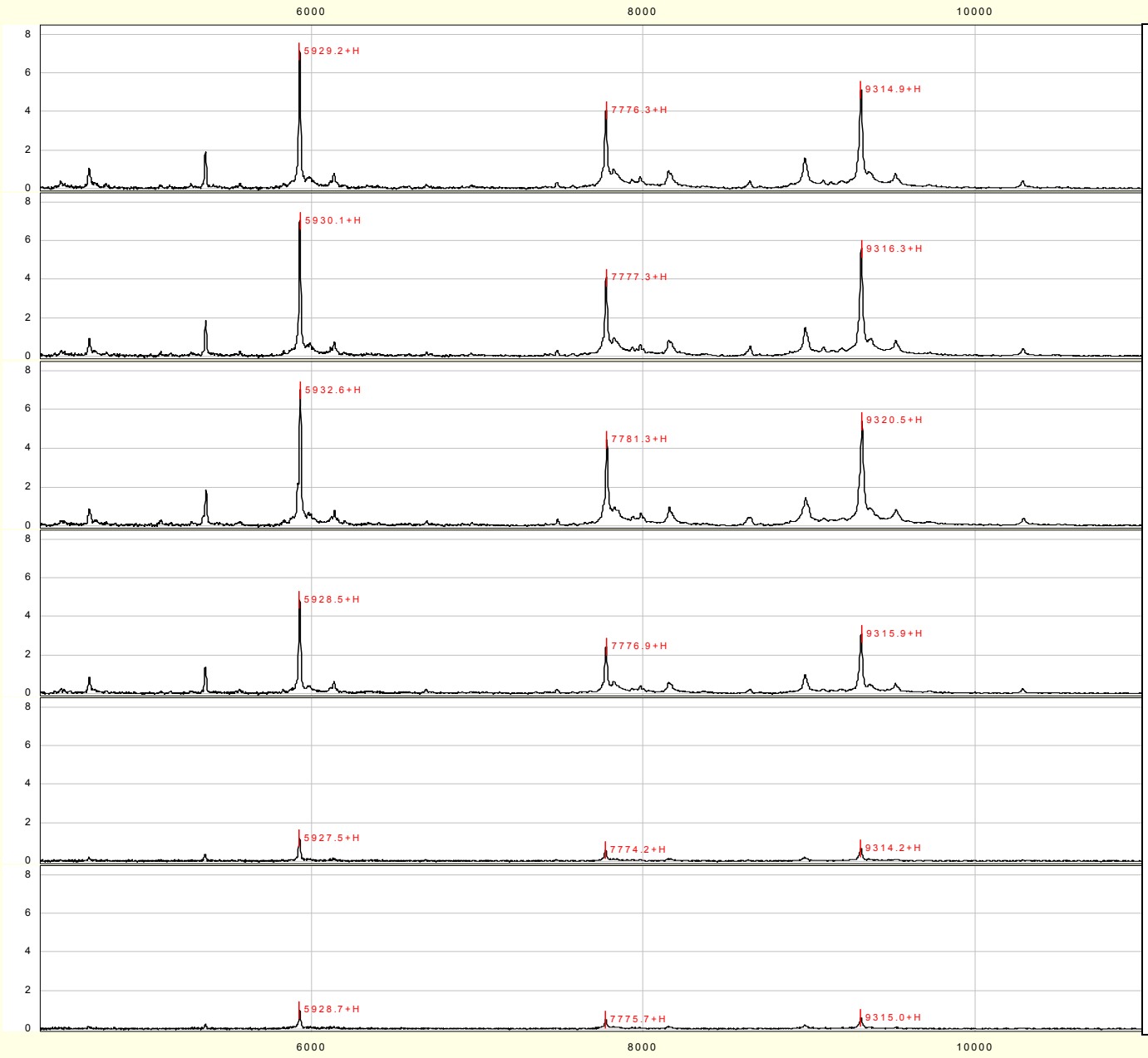
- High Concentration Proteins May Block Binding of Low Concentration Proteins: 10,000 Ci of 5500 D Protein vs. 10 Ci of 7500 D Protein both with Same Binding Characteristics

SELDI ANALYSIS

Protein Chip

- All Proteins/Peptides Bound to Chip May Not Be Released/Ionized.

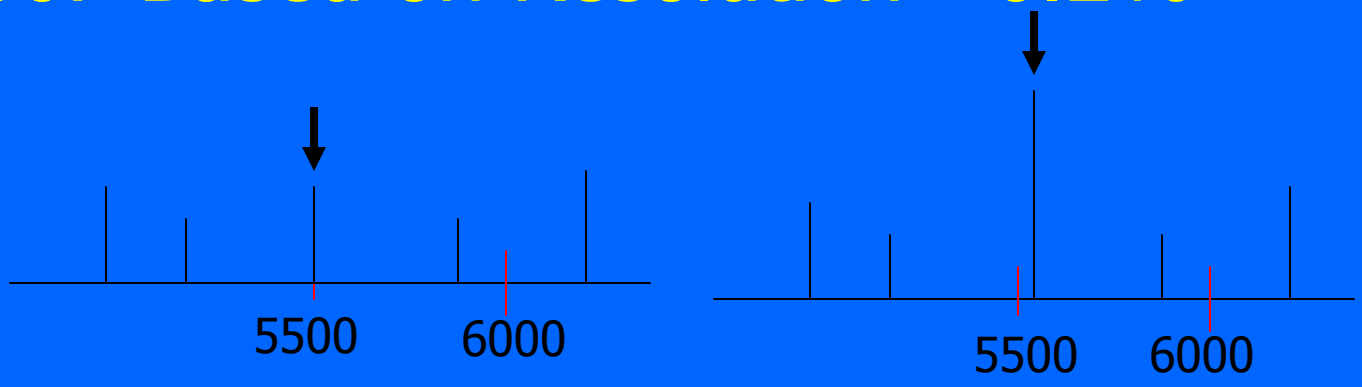
QC Sample Spectra at Multi-times Reading



SELDI ANALYSIS

Spectrum

- "Directed" and "Non-Directed" Approaches to Begin Spectral Analysis
- Directed= Peak at 5500 Same As Peak at 5507 Based on Resolution +0.2%



SELDI ANALYSIS

Spectrum

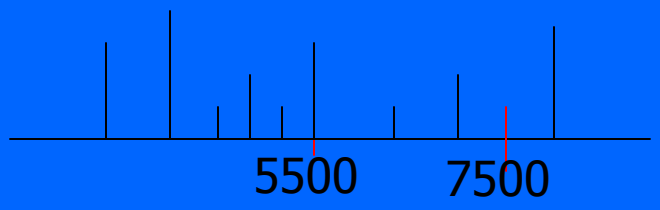
- Primary Peaks- Disease Has Unique or Larger Peak than Non-Disease; Thus, the Disease Produces a Molecular Product.

- Secondary Peak-Disease Causes a Decrease in Molecular Species Normally Present via Change in Metabolism or Excretion and/or Shutdown in Production

SELDI ANALYSIS

Spectrum

- Components of Spectra at Molecular Weights of Less Than 20,000 May Represent Metabolites of Proteins/Peptides Rather Than Intact Proteins/Peptides

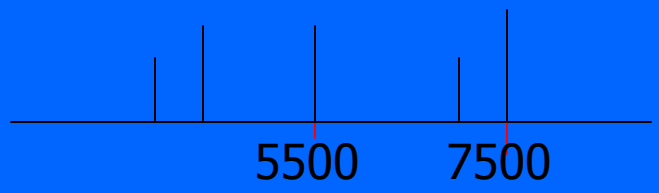


SELDI ANALYSIS

Spectrum

- Peaks May Not Provide Independent Information: For Example the Peak at 5500 D May Be A Metabolite of the Peak at 7500

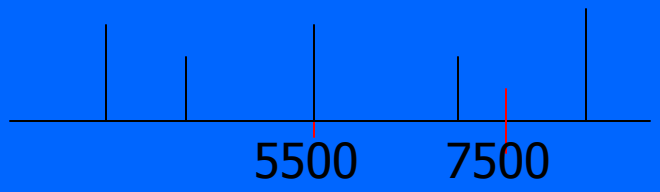
7500 ⟶ 5500



SELDI ANALYSIS

Spectrum

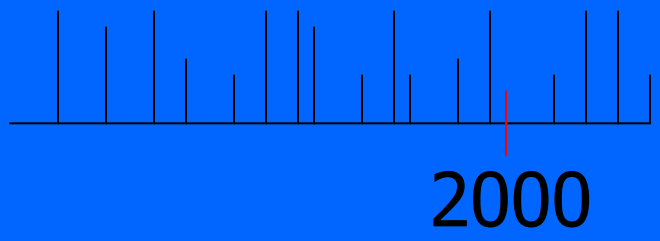
- A High Concentration Protein May Prevent Identification of Low Concentration Protein: 1200 Ci of 5500 D Protein vs. 100 Ci of 5510 D Protein Even with Different Binding Characteristics to Same Chip



SELDI ANALYSIS

Spectrum

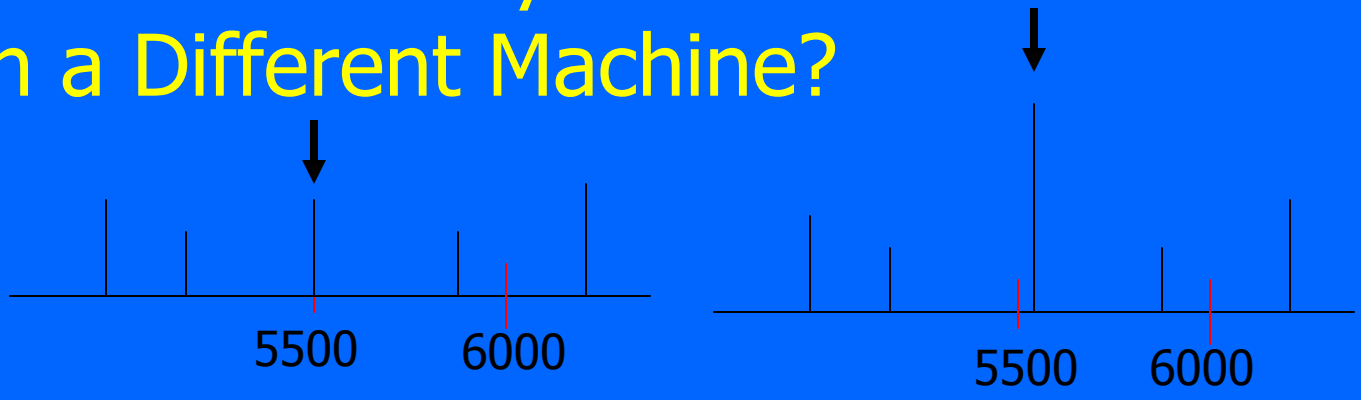
- All Areas of the Spectrum Are Not The Same
  - Molecular Weights of Less Than 2000
    - No Standards; Noise; Contamination
  - Weights of Greater Than 50,000
    - Proteins of High Concentration



SELDI ANALYSIS

Spectrum

- How Variable Is The Peak Location and/or Amplitude When the Same Sample Is Run
  On the Same Day on the Same Machine?
  On the Next Day on the Same Machine?
  On a Different Machine?



SELDI ANALYSIS

Spectrum

- Eastern Virginia Medical School; UAB; U of Texas San Antonio; U of Pittsburgh Medical Center; Johns Hopkins Medical Center; Uniformed Health Services
- ***All Were Able To Standardize Their Machines and To Obtain Comparable Data on 14 Cancer and 14 Non-Cancer Cases***

SELDI ANALYSIS